CHAPTER 1

INTRODUCTION

1.1 Overview of human hair samples

In the last few decades the analytical study of trace elements in biological and human organs has become very important. Occupational diseases, poisoning, and environmental diseases are accurately diagnosed by trace elemental analysis of human materials and the state of health can be characterized with the analytical results. The importance of these examinations is proved that there are several trace elements in the human body that are important in the biochemical processes. An excess of the toxic elements causes serious problems in the body. Thus, it is very important to examine the trace element concentrations in the body. Until now, the concentrations of trace elements in human body have been determined by the analysis from blood, serum, urine or plasma. The analysis of blood is accompanied by several problems, whereas the analysis of human hair is useful in monitoring the levels of certain trace elements in the body. Thus, scientists were looking for a new human biological sample which has the above-mentioned problems could be eliminated. In 1954, Flesch proposed the use of hair samples for trace element analysis, and Hammer *et al* [2] proved that in some cases hair analysis was better than blood analysis. Human hair has been demonstrated as a major of vehicles to excreted substances from the human body. In comparison, the concentrations of heavy metals are found within hair samples higher than the levels found within blood or urine samples up to ten-fold. Hair samples also give information about the intracellular accumulations of trace elements.

In recent years, human hair has been attracted the interest of researchers from the environmental and forensic science increasingly. Human hair has been used as an evident in suicide, poison and homicide police cases to prove that the cause of death is from poison or not. Because the toxics of heavy metal can accumulate within hair for a long time [1-3]. Human hair has been used as:

(i) screening tool in medical practice

(ii) assessing as the nutritional and toxicological status of the body

(iii) evaluation of heavy metal poisoning

(iv) monitoring environmental or occupational exposure

(v) forensic sample

The advantages of human hair samples

The use of human hair samples which favored for the determination of heavy metals can be summarised as follows [4-6]:

(i) sampling is carried out easily and painlessly

(ii) human hair samples can be collected more quickly and easily than samples of blood, urine or any other tissue

(iii) human hair is a stable matrix, samples can be stored at room temperature for a long time and it not necessary to have special storage conditions

(iv) hair is an inert and chemically homogeneous sample

(v) the composition of hair does not change measurably

(vi) most of the toxic elements are accumulated within hair higher than in other human organs

(vii) repeated determinations can be operated easily over time

(viii) serum and urine concentrations provide both an acute index and also over a relatively short time period whereas the concentrations in hair provide a retrospective index of trace element supplies

(ix) hair provides information on the trace element concentrations of the intracellular space

(x) with the knowledge of the growth rate of the hair, it is possible to select the studied previous period (usually 2–3 months)

1.2 Cadmium

Generally, cadmium is a by-product of zinc production because cadmium occurs as a minor component in most of zinc ores. However, traces do naturally occur in phosphate, and have been shown to transmit in food through fertilizer application. General properties of cadmium are shown in **Table 1.1**.

Table 1.1 General properties of cadmium

General properties of cadmium	
Symbol	Cd
Atomic number	48
Atomic weight	112.411 g/mol
Electron configuration	[Kr] 5s2 4d10
Crystal structure	Hexagonal
Occurrence	Bluish-white metal
Melting point (°C)	321.07
Boiling point (°C)	767

1.2.1 Characteristics

Cadmium is a soft, malleable, ductile, toxic, bluish-white bivalent metal. The most common oxidation state of cadmium is +2 and it is similar in many respects to zinc but forms more complex compounds. Cadmium burns in air to form brown amorphous cadmium oxide (CdO). The crystalline form of the same compound is dark red and changes color when heated, similar to zinc oxide. Cadmium was used for a long time as pigment and for corrosion resistant plating on steel. Cadmium compounds were used to stabilize plastic. It is also used in nickel-cadmium batteries. However, the use of cadmium is generally decreasing in all other applications due to the high toxicity and carcinogenicity of cadmium, the associated health and environmental concerns [7].

1.2.2 Occurrence

Cadmium occurs in trace amounts in a range of plants and animal tissues. In healthy human adults all tissues except kidneys and liver appear to contain about 0.2-0.8 ppm on a fresh basis. This heavy metal appears to accumulate in the kidneys and to some portion in the liver. The external contamination of cadmium is accumulated in the kidneys during lifetime, and if it complexes with zinc enzyme, displacing zinc, cadmium can induce diseases may occur as renal or hepatic zinc deficiencies. In the first place of interest in cadmium toxicity, mild symptoms of poisoning were reported to be produced by 15 ppm of cadmium in foods. Where the concentration of cadmium in the range of 300-400 ppm may intake, cadmium is hoarded in the lungs and then distributed throughout the body, accumulating in the

liver and kidneys. Due to slow excretion, these high tissue levels may remain for several years [8].

1.2.3 Sources of cadmium

Anthropogenic sources of cadmium to the environment are:

(i) distillation and utilization

(ii) copper and nickel smelting

(iii) fossil fuel combustion.

Natural sources of cadmium to the atmosphere are:

(i) volcanic activity

(ii) forest fires

(iii) wind blown transport of soil particles

In fact, the anthropogenic sources of cadmium increase 3–10 times more cadmium to the atmosphere than natural sources. Other sources of concern are phosphate fertilizers, which may contain high concentrations of cadmium depending on the origin of the rock, and the application of contaminated sewage sludge as a soil improvement. The majority on occupational exposure appears increasingly in non-ferrous metal smelters, in the production and processing of cadmium, its alloys and compounds and in the recycling of electronic waste. Non-occupational exposure is mainly occurs from cigarette smoke which contains relatively high concentrations of cadmium. But for non-smokers who are not occupationally exposed, nutrition is the main route of exposure to cadmium [9].

1.3 Lead

Lead is the commonest of the toxic metal elements. Metallic lead is occurred in nature, but it is abbreviated. Lead is usually found in ore with zinc, silver and copper. The main-group element of lead mineral is galena (PbS), which contains 86.6% lead. Other common varieties are cerussite ($PbCO_3$) and anglesite ($PbSO_4$). General properties of lead are shown in **Table 1.2**.

Table 1.2 General properties of lead

General properties of lead	
Symbol	Pb
Atomic number	82
Atomic weight	207.2 g/mol
Electron configuration	[Xe] 4f14 5d10 6s2 6p2
Crystal structure	Cubic
Occurrence	Bluish-white metal
Melting point (°C)	327.46
Boiling point (°C)	1,749

1.3.1 Characteristics

Lead is bright and silvery when freshly cut but the surface rapidly tarnishes in air to produce the more observed dull luster. It is a dense, ductile, very soft, highly malleable, bluish-white metal and it has poor electrical conductivity. The prominent property of lead is highly resistant to corrosion, therefore it is usually used to contain corrosive liquids such as sulfuric acid. Because lead is very pliable and

resistant to corrosion it is extensively used in building construction. Lead can be hardened by addition a small amount of antimony or other metals. All lead, except ^{204}Pb, is the end product of a complex radioactive decay. Lead is also poisonous as are its compounds.

1.3.2 Occurrence

Most of human and animal tissues contain significant amounts of lead, but little is known about this metal in the tissues. It presumably occurs as a contaminant. Lead is a poisonous element and there are extensive data on lead toxicity. Lead poisoning obviously affects prophyrin metabolism, in fact, intake of lead more than 50 mg per day always excrete prophyrin in urine. Lead is absorbed slowly as well as incompletely from the gastrointestinal system, and can be absorbed from the respiratory system when inhaled. Normally, lead poisoning is chronic because the metal is excreted even more slowly than it is absorbed. Consequently, lead in exposed individuals tends to accumulate in the body and is stored by the tissues, especially in the bones. Symptoms of poisoning may follow such storage when, owing to other diseases, the stored lead is released into the general circulation [8,10].

1.3.3 Sources of lead

(i) Paint

Lead was used in paint to mix in color for improving the ability of the paint to hide the surface it covers, and to make it durable.

(ii) Dust

Lead dust is the most common way that people are exposed to lead. Most of lead dust comes from whittling and flaking paint or when paint is abraded, sanded, or disturbed during home remodeling. Chipping and peeling paint is mostly found on surfaces that scrape or bump up against another surface.

(iii) Soil

Soil can be contaminated with lead due to the decadent of lead-based paint on buildings and playground equipment. The soil near roads or highways may contain high lead levels that accumulated from years of toxic fumes and pollution from cars which used leaded gasoline.

(iv) Drinking water

Lead contaminates into drinking water from the corrosion or wearing away of materials containing lead in the water distribution system, household or building plumbing. These materials include lead-based solder used to join copper pipe, brass and chrome plated brass taps.

(v) Work place

People exposed to lead at work may bring lead home on their clothes, shoes, hair, or skin. There are many more jobs that exposed people to lead include home improvement, painting and refinishing, car or radiator repair, plumbing, construction, welding and cutting, electronics, municipal waste incineration, battery manufacturing, lead compound manufacturing, rubber products and plastics manufacturing, working in brass or bronze foundries, demolition, and working with scrap metal.

(vi) Food supply

Lead can be found in candy, wrappers, pottery containers and in certain ethnic foods. The lead ink from the paper wrapper may contaminate the candy. After the can has been opened, lead gets into the food especially if the products are acidic food cause lead get into the food faster.

(vii) Cosmetics

Some cosmetics contain lead such as creams, powders and pastes were used to hide defects, enhance beauty, decorate as a creative expression, preserve beauty, and combat the ravages of time and exposure. Lead exposure primarily via hand-to-eye-to-mouth movement and subsequent ingestion of particles [11].

1.4 Microwave-assisted digestion

The application of microwave ovens began to widely use in chemical laboratories since the late 1980's. Microwave units have become increasingly popular because of the significant improvement in chemical reaction rates that are possible using microwave radiation. Microwave-assisted digestion are well established and accepted as a fast alternative for sample preparation, because microwave-assisted digestion uses a short period of time to decompose material comparing to classical digestion technique from days or hours to minutes. Microwave power is an extremely useful auxiliary factor, the procedures are accomplish using especially designed cavity-microwave ovens or focused-microwave ovens with respective reaction vessels. Both can be performed properly when applying tailored methods for different types of organic and inorganic samples as follow:

(i) The cavity-microwave oven

The cavity-microwave oven uses high pressure closed vessels and is proper for dealing with masses of organic samples lower than 0.50 g to avoid excessive pressure build-up and undesirable effects. The procedures are suggested for trace analysis to avoid losses and memory effects even when working at high temperature and pressure.

(ii) The focused-microwave oven

The focused-microwave oven is attractive to use for digestion of large masses organic samples, which employs open vessels and digestion is achieved at atmospheric pressure. During the digestion, the vessels are not open, instead, there is a purge system to remove the gaseous products which produced during the oxidation of the sample. The system is advantageous due to its capacity to allow the removal of gases generated during the digestion process. But losses of volatile elements when applying focused-oven may occur as a result of the reaction vessel design [12].

1.4.1 Principle of microwave-assisted digestion

Microwave-assisted digestion is used microwave energy to heat solvents in contact with solid samples or liquid samples and to partition analytes from sample matrix into the solvent. The principle of microwave-assisted digestion is based on the direct effect of microwaves on molecules of the digestion system caused by ionic conduction and dipole rotation mechanism. Microwave-assisted digestion differ from usual forms of classical digestion, because microwaves heat the digestion system directly and leading to shorten the consumption of digestion times. The ability of a

solvent to absorb microwave energy partly depends on the dissipation factor as follow:

$$\tan \delta = \frac{\varepsilon''}{\varepsilon'}$$

Where $\tan \delta$ = the dissipation factor

ε" = dielectric loss (a measure of the efficiency of converting microwave energy into heat)

ε' = dielectric constant (a measure of the polarizability of a molecule in an electric field)

Generally, polar solvents and ionic solutions especially acids are strongly absorbed microwave energy, but non-polar solvents do not heat when used microwave-assisted digestion.

1.4.2 Microwave-assisted digestion procedure

The procedure of microwave-assisted digestion may occur through any one or several of the following mechanisms:

(i) to extract analyte into a single solvent or mixture of solvents that strongly absorb microwave energy

(ii) to extract analyte into a combined solvent that contain both high and low dielectric losses mixed in various proportions

(iii) to extract analyte with a microwave transparent solvent from a sample of high dielectric loss

Microwave-assisted digestion may be carried out by pressurized (closed) system or focused (opened) system where a solvent and sample are placed and then exposed to microwave energy.

1.4.3 Influential parameters

The main parameters influencing microwave-assisted digestion performance include:

(i) type of solvent

(ii) volume of solvent

(iii) microwave power and digestion time

(iv) temperature

Usually, organic solvents are mostly used for digesting organic or bioactive compounds. Selection of the solvent should be considered mainly to microwave-absorbing properties of the solvent, selectivity towards the analyte and interaction of the solvent with the sample matrix. However, most applications of microwave-assisted digestion have involved mixtures of a polar solvent and water, including the humidity of biological matrices. Normally, in conventional digestions, the increasing of solvent volume will increase the recovery of the analyte. But, in microwave-assisted digestion, the higher volume of solvent may conduct to the lower recovery, which may be caused by the insufficient stirring of the solvent in the microwave system. The volume of solvent using in microwave-assisted digestion depends on the size and the type of sample, which may be about 10 times less than those used in classical digestions. The suitable power and exposure time used in microwave depend on the type of sample and solvent. In theory, microwave-assisted digestion should be

used high-power of microwave to reduce exposure time as much as possible. However, a very high-power of microwave may reduce the digestion efficiency through degrading the sample or rapidly boiling of solvent in focused systems. Generally, digestion times in microwave-assisted digestion are much shorter than those of classical digestion. Usually, increasing of digestion times above the optimal range does not improve digestion efficiency, and in some cases may be decreased the recovery of analyte such as thermolabile compounds. In most cases of microwave-assisted digestion, increased of temperatures leads to improve digestion efficiency. But application of thermolabile substances should be considered to the loss of analyte at high temperatures [13].

The advantages and disadvantages of microwave-assisted digestion [14]

There are many advantages including some disadvantages of microwave-assisted digestion as shown in **Table 1.3**.

Table 1.3 The advantages and disadvantages of microwave-assisted digestion

Advantages	Disadvantages
(i) Achieve higher temperature (ii) Digestion time greatly reduced (iii) Less reagent and sample usage (iv) Reduction in contamination (v) Reduction in the loss of volatile species	(i) High cost (ii) Limited sample weight

1.5 Cloud point extraction (CPE)

The determination of a very low concentration of trace elements in complex matrixes has often been a problem for analytical chemists. Separation and preconcentration can solve this problem by offer the ability to isolate the target analytes from the matrix solution, including to control or even eliminate the interferences. The classical liquid–liquid extraction and separation methods are usually time consuming, labor extensive and also require relatively large volumes of high purity solvents. Another concern is disposal of the solvent used that creates a severe environmental problem. Cloud point extraction (CPE) is an attractive technique that very useful for the separation and preconcentration of metal ions in biological samples. CPE needs low volumes of the sample (only 10 ml), it also reduces the consumption of solvent, disposal costs and extraction time.

CPE has been used for the extraction and preconcentrations of metal ions for 30 years. In 1976, Watanabe and his co-workers [16] introduced CPE as an encouraging new separation and extraction technique as an alternative to organic solvents. Even though, CPE was firstly introduced for the preconcentration of metals in the form of their hydrophobic complexes, it was extensively exploited as a primary isolation step for the purification of proteins. Numerous studies has been reported including its theoretical background and particularly proposed extraction and preconcentration schemes for the determination of organic and inorganic analytes. A synopsis of CPE capability was presented in 1982, while another attempt to review the literature widely appeared in 1985. Since then, several changes to the classical approach have been proposed, while automation has also been introduced [15-18].

1.5.1 Theory of cloud point extraction

In cloud point extraction (CPE), the role of extraction solvent is performed by a micelle (surfactant-rich phase) occurring from a surfactant solution that is added into the sample. Micelles are surface-active agents (surfactants, detergents) which consist of hydrophobic and hydrophilic moieties in the molecule. A polar or ionic group in micelle is connected to a long hydrocarbon tail (may be linear, branched or containing aromatic rings). Micelles can aggregate in aqueous solution to form colloidal-sized clusters and orientates its hydrocarbon tails towards the center to create a non-polar core. The binding site of surfactant micelles extracting the analyte is shown in Figure 1.1. This is ascribed to their formation consisting of a hydrophilic surface and a hydrophobic core. The hydrophobic core can entrap and thus isolate hydrophobic substances. This ability has been extensively used in the past few years under the term cloud point extraction for the preconcentration of hydrophobic compounds. In addition, under certain conditions these areas also can interact electrostatically with amphoteric or even charged substances such as metal ions [13,16,19].

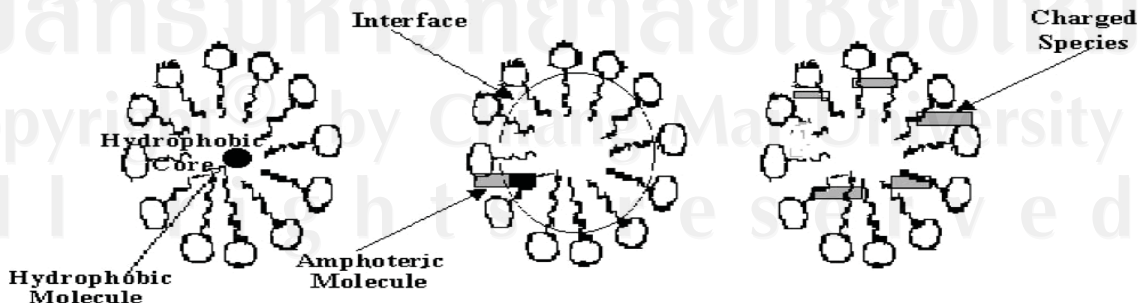

Figure 1.1 Binding sites of a micelle for hydrophobic and amphoteric molecules [19]

Hydrophobic compounds or a large number of bioactive compounds which present in the aqueous solution are favorably partitioned in the hydrophobic core of micelles. In aqueous solutions, low concentrations of surfactant molecules are presented, mainly as monomers although dimers and trimers. Separation of a surfactant–rich phase and an aqueous supernatant phase in CPE, when the minimum concentration of surfactant increases above a certain threshold and occurs the phenomenon is called the critical micelle concentration (CMC). Then, surfactant monomers spontaneously accumulated to form colloidal-sized clusters, known as micelles. However, the shape of micelles depends on the specific surfactant and solution conditions because micelles can be adapted in various shapes. The surfactant concentration close to the CMC usually requires appropriate experimental conditions depending on the nature of the surfactant [13,16]. Micelles can be adapted a variety of shapes as show in **Figure 1.2**.

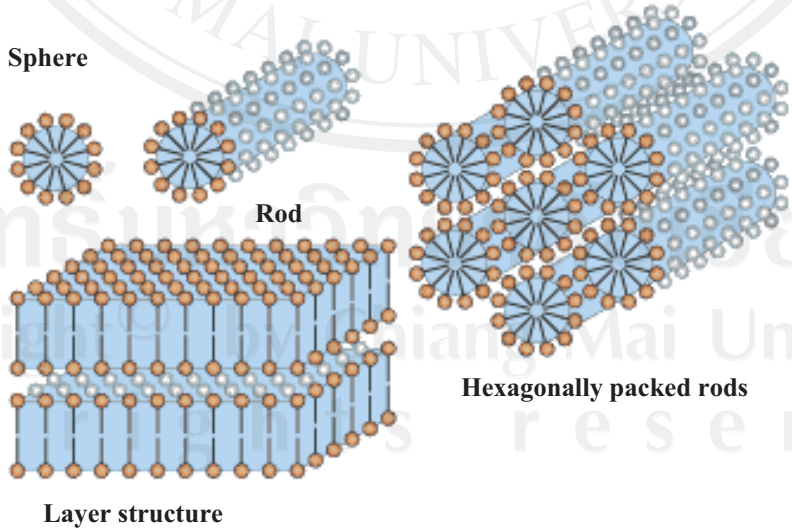

Figure 1.2 Schematic representation a variety of micelle shapes

Regardless of their shape or size, hydrophobic and covalent compounds which present initially in the aqueous solution are favorably partitioned in the hydrophobic core of micelles. The whole process is similar to traditional liquid–liquid extraction (LLE), the only difference being that the organic phase is generated within the aqueous phase and converting a previously homogeneous solution to heterogeneous one by simply gathering its previously scattered hydrophobic suspensions. When the solution conditions such as temperature, pH, the concentration of surfactant, ionic strength and others are appropriated, two phases separation of a surfactant-rich phase and an aqueous phase are occurred in the solution [16, 20]. In other words, the principle of CPE is based on the property of surfactant in aqueous solutions to from micelles and become turbid when heated to the temperature known as cloud point temperature. Above the cloud point, the micelle solution separated into a supernatant aqueous phase and a surfactant-rich phase at the bottom of the test tubes, in which the surfactant concentration is closed to CMC. Any analyte that solubilized in the non-polar core of the micelles will be separated and become concentrated in the small volume of surfactant-rich phase [21].

1.5.2 The advantages of cloud point extraction [16]

(i) CPE is simple, rapid and sensitive methodology for the preconcentration of metals from various samples

(ii) the capacity to preconcentrate an excess of analytes with almost quantitative recoveries

(iii) the preconcentration factors obtained are comparable or superior to other schemes, and they can also be modified on demand by varying the amount of surfactant

(iv) commercial surfactants are cost effective and friendly to environmental

(v) the amounts of reagents used in CPE are minimal compared to the amounts of organic solvents used in conventional extraction

(vi) the mild conditions applied in CPE allow for preconcentration schemes targeted at thermally sensitive analytes such as molecules of biological and environmental interest

(vii) the surfactant-rich phase is compatible with most mobile phases used in hydrodynamic analytical systems, also it increases atomic signal in FAAS and wettability of the graphite surface in ETAAS techniques

In terms of reproducibility and credibility of CPE, handling the surfactant-rich phase is a critical feature. Usually, after phases separated by centrifugation, the test tubes are cooled in an ice bath so that to increase the viscosity of the surfactant-rich phases which adhere at the bottom of the test tubes. Aqueous phases which in the supernatant phase can be removed simply by a syringe. In suggestion, complete removal of water traces can be achieved by evaporation under a nitrogen, argon or helium stream. An important gain of CPE is that obtained surfactant-rich phase is reduced to a minimum volume, approximating its initial volume, which rarely exceeds 100 μl per 10 ml of sample volume. However, there are several limitations inherent in CPE in using of the surfactant-rich phase obtained. Due to its viscosity, it cannot be injected directly to classical analytical instruments. Thus, it has to be diluted with an

aqueous or organic solvent to reduce its viscosity, which maybe decreasing the anticipated theoretical preconcentration factors. Recently, there have also been reported to use zwitterionic surfactants or non-ionic surfactants in CPE, although the slurry-like nature of the surfactant-rich phase obtained and the difficulty of using the surfactant-rich phase are often mentioned [22-24].

1.5.3 Phase separation behavior of surfactants

Upon heating in CPE procedure, aqueous solutions of many non-ionic surfactants become turbid at the cloud point temperature, and above this temperature there is a separation of the solution into two phases. Phase separation occurs from the competition between entropy (which favors miscibility of micelles in water) and enthalpy (which favors separation of micelles from water). The variation of these two contributions depending on temperature, either a lower (non-ionic surfactants) or an upper (switterionic surfactants) consolute point can be resulted. For non-ionic systems, phase separation typically occurred over a narrow temperature range because the turbidities of the system stems are from the presence of very large surfactant aggregates that scattered the visible light passing through the solution. The phases consist of an aqueous phase and a surfactant-rich phase that appears only in the vicinity of the cloud point temperature. In the aqueous phase typically equals or exceeds the CMC, the micelles or other surfactant aggregate species are also presented. The actual physical separation of the phases is facilitated by the difference in density between the two phases (aqueous phase and surfactant-rich phase). The phase separation process is reversible and upon cooling the mixture to a temperature below the cloud point, the two phases again merge to form a homogeneous solution.

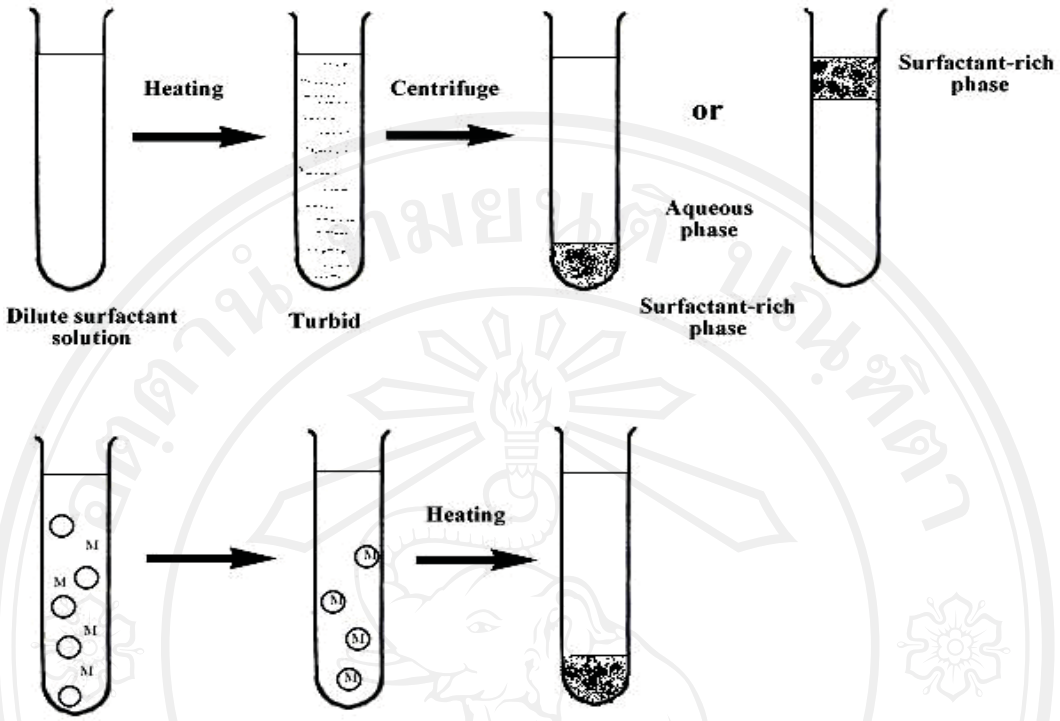

Figure 1.3 Pictorial representation of steps involved in the phase separation and CPE of an analyte [25] (This figure is modified from F. H. Quina and W. L. Hinze, 1999)

In **Figure 1.3**, the non-ionic or zwitterionic surfactant is added to the aqueous solution containing the analyte to be extracted and analyzed. In the top of the pictorial representation has shown the steps of the phase separation. Upon heating above the cloud point temperature, a non-ionic surfactant solution will become turbid and after a certain time of centrifugation, the two phases will be separated. In the bottom of the pictorial representation has shown the cloud point extraction of the analyte. Addition of a non-ionic surfactant above its CMC will be formed of micelles (represent by the circle) that can interact with and bind analyte (represent by M). After temperature alteration and centrifugation, the micelles-bound analyte will be separated from the

original aqueous phase and become concentrated in the surfactant- rich phase, which can be analyzed or subjected to further treatment as required [25].

1.5.4 Influential parameters

In CPE, to achieve such a wide range of applications, several chemical parameters have to be optimized to achieve the quantitative extraction. For organic species, the parameters which susceptible to the optimization stem are the properties of the surfactant medium. However, for inorganic species, where the quantitative formation of a hydrophobic complex is an essential prerequisite for efficient of CPE, properties of the surfactant medium have to be optimized more carefully, considering the variables of complex formation. Ordinary parameters for both organic and inorganic species, which have to be optimized to make CPE successful are:

1.5.4.1 pH of the solution

For organic species, pH is the most important factor to partition the target analyte in the surfactant-rich phase. Especially for ionizable species such as phenols and amines, maximum extraction efficiency is achieved at pH values where the uncharged form of the target analyte predominates. In recently development of CPE, schemes based on ionic surfactants were used effectively to extract charged analytes. However, for inorganic species, there was little differentiation in the extraction efficiency of the complexes formed at different pH values, because these complexes are bulky, uncharged and covalent. In any other case, the role of pH is the same as in traditional pH-selective fractional precipitation, where the separation of several metal ions is made practicable by repeatedly adjusting the pH.

1.5.4.2 Effect of surfactant concentration

The concentration of surfactant is an important factor which has to discuss on CPE. There is a narrow range of surfactant concentration which accomplished the easy phase separation, maximum extraction efficiency and analytical signal. Increasingly, outside this optimal range, the analytical signal is observed to deteriorate due to the increase in the final volume of the surfactant-rich phase that causes the preconcentration factor (phase–volume ratio) to decrease. However, if surfactant concentration is decreased from that recommended, accuracy and reproducibility would probably suffer because the resultant surfactant-rich phase would not be sufficient to make reproducible measurements of extraction and separation.

1.5.4.3 Selection of the chelating agent

Selection of the chelating agent is the controlling factor for extracting all metal in CPE schemes. Since Watanabe's pioneering application of CPE in metal extraction, several chelating agents have been utilized in order to produce sufficiently hydrophobic complexes to be isolated in the surfactant-rich phase of a micelle solution. Based on their reactivity and formation constants with the target metal species, some of the most widely applicable reagents are carbamates, pyridylazo, quinoline, and naphthol derivatives. These molecules are universal chelators that form hydrophobic compounds with the majority of metal ions and they can be applied when an element-specific detector is available. Other reagents, such as *O,O*-diethyldithiophosphate, have been utilized for more specific applications. In any case, a ligand is selected with the requirement that the derived complex is sufficiently

hydrophobic, possesses a high partition coefficient, formed quickly and quantitatively with the least possible excess. The thermodynamics parameters such as formation constant (K_f), as well as the kinetics of complex formation and transfer into the micelle phase control the whole procedure, while the contributions of cloud point and micellization parameters are less mentioned. The distribution behavior of metal chelates in the surfactant medium is depended on the nature of the complex and the prevailing conditions. In contrast with organic solvents, the distribution constants are almost independent of the nature of the metallic ions [26].

1.5.4.4 Equilibration temperature and complexing time

Temperature and complexing time of the CPE procedure are important factors to complete reactions, and to achieve easy phase separation. Normally, temperature change results in two-phase separation of non-ionic and zwitterionic surfactant solutions, while other parameters are involved in two-phase separation process of ionic surfactants. The parameter inducing phase separation can limit the types of compound that can be extracted. Thus, CPE at high temperatures cannot be applied to analysis of thermolabile compounds, while acidic solutions are not suitable for weak basic compounds. In addition, high temperatures are not suitable in the proposed analytical method because they could create stability problems for chelates and chelating agents. Therefore, if high temperatures are not required by the experimental conditions, they can be avoided, even if they may give some improvement for extraction efficiency.

However, it has been proved that when dealing with such inert metals or metal complexes, it is necessary to apply elevated temperatures higher than 80°C and

prolonged incubation times in order to achieve satisfactory extraction. This observation comes as affirmation of the belief that the whole procedure is controlled by the requirement that the reaction should be completed for complex formation to be efficient, because clouding and phase separation are occurred even at room temperatures. As a universal observation, temperature seems to play an additional role in enhancing preconcentration efficiency and enhancement factors, as it is reported that applying elevated temperatures leads to dehydration of the micelle and increasing the phase volume ratio. An important point with considered to complexing time is that, for metals, their reaction with chelating agents and their transportation inside the micelle are kinetically controlled. Although, thermodynamics are favored but it can simulated the shift of equilibrium towards precipitation. Therefore, it is essential to maintain the reaction time above a minimum threshold for quantitative extraction. In most studies, a reaction time of up to 10 minutes is considered to be sufficient [13, 27].

1.5.4.5 Effect of ionic strength and centrifugation

Ionic strength and centrifugation time have also been of consideration, although they have proved to have a negligible effect on the performance of CPE. Increasing ionic strength enhances phase separation through sorting out phenomena that also apply to conventional extraction schemes, yielding higher recoveries without by any means deteriorating the analytical performance. In that direction, it is possible to apply this factor directly to difficult matrixes such as water in environmental and biological fluids. Generally, centrifugation time hardly ever affects micelle formation but urges phase separation in the same sense as in conventional separations of a

precipitate from its original aqueous environment. Usually, centrifugation times around 5–10 min are efficient for most CPE procedures.

1.5.5 Implementation of CPE in metal analysis

Cloud point extraction used for metal determinations is relatively simple. A few milliliters of concentrated surfactant solution are added into the aqueous sample, this volume is in the range of tens of hundreds of milliliters. When necessary, a chelating agent solution is dissolved in an organic solvent or directly in water, depending on its solubility. The solution is then heated above its cloud point and separation of the phases usually occurs after centrifugation. The discard of bulk aqueous phase after separation of micelle phase is facilitated after an ice bath, because the viscosity of the surfactant-rich phase is increased. CPE efficiency mainly depends on many factors such as the interaction of metal species with surfactant, the formation of complex when a chelating agent takes part, the kinetics of chelating agent to form complex and the phase transfer in the micelle media. It is interesting that the distribution constants of metal chelates in micelle medium depend on the nature of the metal ions with consequent variations in selectivity. Because of the hydrated nature of the surfactant phase, the distribution mechanisms are different from those of conventional solvent extraction, where the distribution constants of chelates are almost independent of the nature of the metal ions [27].

Usually, the experimental procedure of CPE can be described as shown in **Figure 1.4** [16, 28-29].

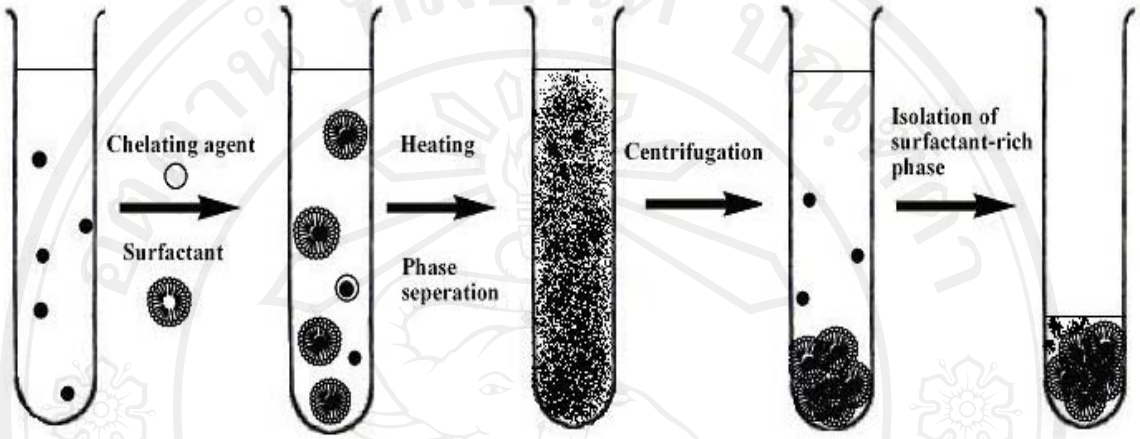

Figure 1.4 The experimental procedure for CPE in metal analysis [16]

(This figure is modified from E.K. Paleologos, D.L. Giokas and M. I. Karayannis, 2005)

(i) the metal reacts with a suitable complexing agent to form a hydrophobic complex

(ii) clouding is generated by increasing temperature above the cloud point

(iii) the micelles formed entrap the metal complexes inside their hydrophobic core

(iv) the surfactant-rich phase is subsequently separated from the bulk aqueous one by centrifugation

(v) isolation of the surfactant-rich phase

1.5.6 The surfactant-rich phase in analytical atomic absorption spectrophotometry

Before measurement the analyte by atomic absorption spectrophotometry (AAS), the solution of the surfactant-rich phase is always necessary to have a low viscosity in order to compatible with the requirement of the nebulizer. However, the presence of methanol and surfactant in the flame of AAS was a subject of some debate in the past. Mainly, it was mentioned that the use of organic cosolvents in the flame could enhance the sensitivity. This phenomenon has been attributed to the low surface tension of organic solvents, which can serious affect the nebulization process. There are reported about the use of surfactants in aqueous solution for the same purpose as organic solvents appreciable improvement in sensitivity in the FAAS signal. It is now more accepted that the formation of small droplets in the nebulizer in the presence of a surfactant can favourably increase their transport efficiency to the flame as well as the efficiency of sample atomization [27, 30-33].

1.6 Atomic absorption spectrophotometry (AAS)

Atomic absorption spectrophotometry (AAS) is used for the qualitative and quantitative analysis of perhaps 70 elements. Since the development of commercial atomic absorption instrumentation in the early 1960s, AAS has been used in chemical laboratory increasingly. AAS can be simply defined as the absorption of radiant energy by atom, and it is the commonest instrumental method for analyzing metal ions and some metalloids. This absorption and its quantitative correlation with the concentration of metal ions initially present in a sample solution. The production of atoms from a chemical compound requires the absorption of energy. This energy is

usually supplied in the form of heat from a flame. After vaporization the compound is partially or wholly dissociated into its elements in the gaseous form and some of these atoms can absorb radiant energy of a characteristic wavelength and become excited to a high energy state. The absorption is measured at one of several characteristic wavelength of the element being determined. The wavelength is selected by a monochromator and measured by a detector. This absorption is correlated with the concentration of metal ions originally presents in a sample solution [8,34].

1.6.1 Theory of atomic absorption spectrophotometry

Atomic absorption follows an exponential relationship between the intensity (I) of transmitted light and the absorption path length (l) which is similar to Lambert's law in molecular spectrophotometry.

$$I = I_o \exp(-k_v l) \qquad (1)$$

Where I_o = the intensity of the incident light beam

k_v = the absorption coefficient at the frequency v

In quantitative spectrophotometry, absorbance A is defined by:

$$A = \log(I_o / I) \qquad (2)$$

Thus, from equation (1) can obtain the linear relationship:

$$A = k_v l \log e$$

$$= 0.434\, k_v l \qquad (3)$$

Atomic absorption corresponds to the transitions from lower to higher energy states. Therefore, the degree of absorption depends on the population of the lower level [34].

1.6.2 Flame atomic absorption spectrophotometry (FAAS)

Flame atomic absorption spectrophotometry (FAAS) is a very commonest instrumental method for analysing metals and metalloids in environmental samples. FAAS works by introducing the samples into an atomizer where it is dissociated into its constituent atoms. The basic principle of the technique is that a sample material is converted to atoms by a flame atomizer. A hollow cathode lamp constructed from the analyte element is then used to excite electrons in the analyte atoms to a higher energy level. The intensity of the light reaching the detector, therefore, decreases in proportion to the concentration of the analyte. The method is specific for the element of interest although some chemical and physical interferences are still possible. FAAS is relatively inexpensive and simple to operate. It can be used for the analysis of liquid samples only and relatively large sample volumes are required [35-36].

1.6.3 The basic components of FAAS

A schematic diagram of flame atomic absorption spectrophotometer is shown in **Figure 1.5**.

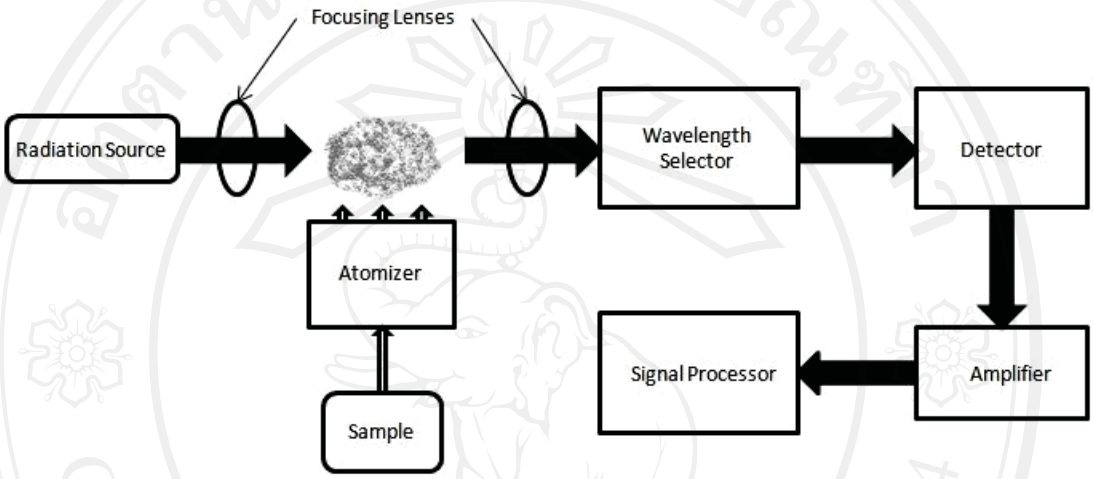

Figure 1.5 The basic components of FAAS [37]

1.6.3.1 The light source

The major requirement of a light source is that it should be emit resonance radiation of the element with a half-life less than width of the absorption line. Normally, the half-width should be around 0.01Å. The most useful radiation source of AAS is the hollow cathode lamp. Hollow cathode lamp has been used for many years as a light source to produce sharp spectrum lines in spectrophotometry, because of the hyperfine structure of the lines produced. It provides the analytical light line for the element of interest and provides a constant yet intense beam of that analytical line.

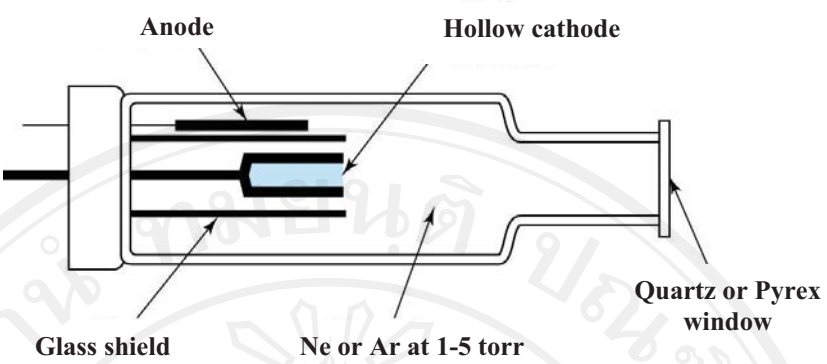

Figure 1.6 Diagram of a hollow cathode lamp [38]

Figure 1.6 illustrates a schematic representation of a hallow cathode lamp. The cathode consists of a hollow cup made from the material of the element to be determined. The anode is a tungsten wire or spherical disk. The two electrodes are housed in a glass envelope containing an inert gas at low pressure. The window or face of the lamp is constructed of quartz or borosilicate glass depending on whether ultraviolet light must be passed. The spectrum intensity depends on several factors such as the efficiency of the inert gas bombardment on the cathode and the kinetic energy of the gas, which controlled by the potential impressed on the electrode and the mass of the atoms. Hollow cathode lamps for about 40 elements are available from commercial sources. Some are fitted with a cathode containing more than one element, such lamp provide spectral line for the determination of several elements. The development of hollow cathode lamp is widely regarded as the single most important event in the evolution of AAS [8, 39-40].

1.6.3.2 The atomizer

The predominant method of atomization for AAS is the use of a nebulizer, spray chamber, burner combination. When the sample solution passes into the flame it must be in the form of small particles. The process of breaking down a solution into fine particles is known as nebulization. The widely used of atomizer is a nebulizer. The function of a nebulizer is to suck up liquid sample at a controlled rate and created a fine aerosol of analyte. Then, mixed the aerosol with fuel and oxidant thoroughly for introducing into the flame.

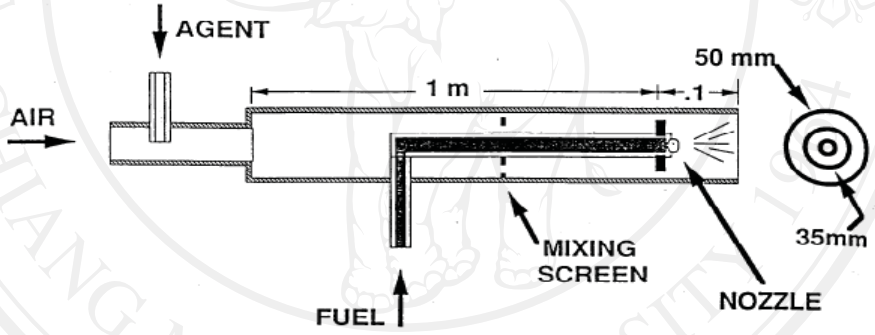

Figure 1.7 Cross-section view of turbulent-flow burner in FAAS [41]

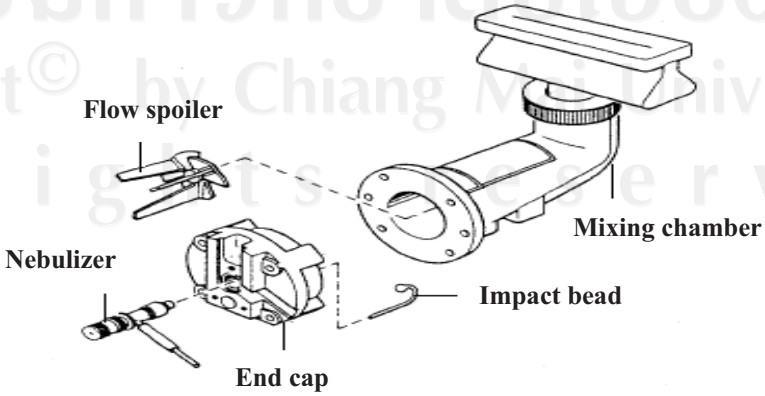

Figure 1.8 Diagram of laminar-flow burner in FAAS [42]

Figure 1.7 is shown a turbulent-flow burner, the nebulizer and burner are combined in a single unit. The sample is drawn up the capillary and nebulized by the flow of gases around the capillary tip. Turbulent-flow burner offers the advantage of introducing a relatively large and representative sample into the flame. Disadvantages include a short path length through the flame and problems with clogging of the tip. Therefore, these burners find little use in present day. **Figure 1.8** is shown a laminar-flow burner. The sample is nebulized by the flow of oxidant past a capillary tip. The resulting aerosol is then mixed with the fuel and flows past a series of baffles that remove all but the finest droplets. As a result of the baffles, much of the sample collects in the bottom of the mixing chamber, where it is drained into a waste container. Then, the aerosol, oxidant and fuel are fed into a slotted burner. Laminar-flow burners provide a relatively quiet flame and a significantly longer path length. These properties tend to enhance sensitivity and reproducibility. Moreover, clogging is seldom a problem [8, 39-40].

1.6.3.3 The monochromator

The basic requirement of a monochromator is to separate the resonance line from other spectral lines nearby. The function of the monochromator is to select radiation of the correct wavelength and eliminate other radiation from the light path. Also, the monochromator is to isolate the absorption line from background light due to interferences and remove scattered light of other wavelengths from the flame. Therefore, monochromator is necessary component of atomic absorption instruments. Monochromator is used in many optical measuring instruments and in other application where tunable monochromatic light is required [8,34].

1.6.3.4 The detector

A detector is to produce an electrical current that is depends on the light intensity. Photomultipliers are used exclusively on commercial equipment. In practice, it is the function of the detector to measure the intensity of radiation before and after absorption by the sample. From this one can calculate how much radiation has been absorbed from the intense beam [39].

1.7 Literature review

The literature reviews for the determination of heavy metals in human hair samples have been reported with various techniques. The widely used techniques that have been reported for the determination of heavy metals in hair are flame atomic absorption spectrophotometry (FAAS), atomic absorption spectrophotometry with electrothermal atomization (ETA-AAS), inductively coupled plasma atomic emission spectrophotometry (ICP-AES), inductively coupled plasma mass spectrophotometry (ICP-MS), neutron activation analysis (NAA), X-ray fluorescence analysis (XRFA), atomic fluorescence spectrophotometry (AFS) and anodic stripping voltammetry (ASV) [4-5].

The atomic absorption spectrophotometry used either in the flame or graphite furnace mode (FAAS and GFAAS) is a powerful analytical tool for the determination of heavy metals in a great number of samples. For cloud point extraction, preconcentration and separation of the metals with different chelating agents are still necessary. There are many chemicals such as *O*,*O*-Diethyldithiophosphate (DDTP), 1-(2-pyridylazo)-2-naphthol (PAN), 1-(2-thiazolylazo)-2-naphthol (TAN) and dithizone have been used as chelating agent. For surfactant reagent including Triton X-100,

Triton X-114 and Tween 80 have been used for preconcentration of trace cadmium and lead from various samples prior to their determinations by FAAS [15, 43-45].

There are some review papers have been reported the use of microwave-assisted digestion in chemical analysis. Microwave power is an extremely useful factor, which has been exploited for increasing the rate of chemical processes for rapid chemical extraction and decomposition. Microwave-assisted digestion has been used for solid sample and liquid sample preparation. Human hair sample can be acid-digested using microwave-assisted digestion by open or closed reaction vessels. The closed-vessels in the cavity-microwave oven uses high-pressure, it is suitable to dealing with organic sample lower than 0.5 g to avoid excessive pressure build-up and undesirable effects. Thus, this method can be reduced the amount of the samples. Also, the reaction of closed-vessels in the cavity-microwave oven can be reduced the loss effects of samples when working at high temperatures and pressures. In addition, the technique of microwave-assisted digestion is very fast in comparison to classical digestion. Moreover, microwave technology has become very important in sample preparation methodology. Therefore, it is reasonable to use microwave-assisted digestion for sample preparation [46].

The literature reviews of cloud point extraction (CPE) for human hair samples have reported about the principle as well as the advantages of CPE. The use of the CPE process has been applied for preconcentration of metals, metal chelates, biomolecules and many types of organic species. The use of CPE as an alternative to other techniques of separation and preconcentration offers several advantages, including low cost, safety and high capacity to concentrate a wide variety of analytes, varying with high recoveries and high concentration factors. From an analytical point

of view, the surfactant-rich phase can be used to separate or preconcentrate different analytes before their determination. Thus, it is necessary to improve and develop the optimum conditions of CPE to get effective methodology [47].

1.8 Research objectives

The main proposes of this research are as follows:

(1) To improve the preconcentration method of cloud point extraction for determination of cadmium and lead in human hair samples.

(2) To determine the amount of cadmium and lead in human hair samples by FAAS.